ORIGINAL ARTICLE

Association between hepcidin, haemoglobin level and iron status in stage 4 chronic kidney disease patients with anaemia

Reza Alipanah Mogadam,[1] Amir Ghorbanihaghjo,[2] Hassan Argani,[3] Ali Nemati,[4] Firouz Amani,[5] Bahman Bashardoust[6]

Abstract

Objectives: To explore the probable association of serum hepcidin and haemoglobin levels with iron and inflammation statuses in patients of chronic kidney disease stage 4 with anaemia.

Methods: The cross-sectional study was conducted at Tabriz University of Medical Sciences, Iran, from March 2011 to October 2012, and comprised patients of chronic kidney disease stage 4 with anaemia. Serum biochemical factors as well as hepcidin, ferritin, interleukin 6, high sensitivity C-reactive protein and iron levels were measured using standard methods. Statistical correlations were established using regression analysis and Pearson's correlation coefficient.

Results: There were 40 patients among whom 15(37.5%) were males and 25(62.5%) were females with an overall mean age of 55.68±14.4 years. There was a significant inverse relationship between hepcidin and haemoglobin levels ($p<0.05$). There were significant correlations between hepcidin with iron status, nutritional and inflammatory markers such as ferritin, Total iron binding capacity, albumin and interleukin 6 ($p<0.05$ each).

Conclusion: Hepcidin had negative correlation with haemoglobin level in stage 4 chronic kidney disease patients with adequate iron stores, which could be effective in the development of anaemia in such patients.

Keywords: CKD, Hepcidin, Iron status, Anaemia. (JPMA 65: 354; 2015)

Introduction

Chronic kidney disease (CKD) is a public health problem and its prevalence is growing all over the world, and the same is the case in Iran.[1] Anaemia is a major complication in CKD patients.[2] The aetiology of anaemia in CKD is multifactorial.[3] The relative erythropoietin deficiency, shortened erythrocyte survival and the erythropoiesis inhibitory effects of accumulating uraemic toxins contribute to the anaemia in CKD.[3] Importantly, CKD patients have several abnormalities in systemic homeostasis of iron, an essential component in the production of red blood cells (RBCs).[3] Poor absorption of dietary iron in CKD patients and failure in the use of iron stores may cause anaemia.[2,3] According to recent researches, hepcidin is one of the main causes of disturbances in iron metabolism in CKD and anaemia.[4,5] It regulates systemic iron balance by decreasing both intestinal iron absorption and iron release from enterocytes, hepatocytes and macrophages leading to hypoferremia and limits iron availability for erythropoiesis.[4] Hepcidin concentration increases in CKD due to inflammation and renal clearance reduction.[5] Resistance to erythropoietin in CKD can be caused by high concentrations of hepcidin and iron limitation.[6] Despite the central role of hepcidin in the metabolism of iron, limited data is available to link hepcidin and anaemia in CKD stage 4 and there are controversial issues in this case.[7]

The current study was planned to assess possible relationships of serum hepcidin levels with haemoglobin levels, inflammation and iron statuses in stage 4 CKD patients.

Materials and Methods

The cross-sectional study was conducted at the Department of Biochemistry, Tabriz University of Medical Sciences (TUMS), Iran, from March 2011 through October 2012, and comprised CKD stage 4 patients in Ardabil hospitals after approval from the institutional ethics committee. The sample size was based on the inclusion/exclusion criteria indicated in earlier studies.[8-10]

Adult patients (≥18 years) with anaemia who had CKD stage 4 with glomerular filtration rate (GFR) 15-29 ml/min/1.73 m2 were included in the study. Anaemia was defined as haemoglobin levels of less than 13g/dl for men and postmenopausal women, and less than 12g/dl for premenopausal women.[11] Subjects with transfusion within the preceding 6 months, myocardial infarction (MI) history within the preceding 3 months, surgical history within the preceding 3 months, malignancy, diastolic

[1,4,5]Faculty of Medicine, Ardabil University of Medical Sciences, Ardabil, Iran, [1]Department of Biochemistry and Clinical Laboratories, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, [2,3]Biotechnology Research Center, Tabriz University of Medical Sciences, Tabriz, [6]Department of Dialysis, Ardabil University of Medical Sciences, Ardabil, Iran.

Correspondence: Amir Ghorbanihaghjo. Email: ghorbaniamir@hotmail.com

blood pressure (DBP) greater than 100mm/hg, uncontrolled diabetes, severe hyperparathyroidism (parathyroid hormone [PTH]>800pg/ml) and consumers of drugs such as angiotensin receptor blockers and angiotensin-converting enzyme inhibitors were excluded.

Following overnight fast, blood samples were obtained from the patients. GFR was calculated by the Cockcroft-Gault formula.[12] Blood haemoglobin (Hb) and haematocrit (Ht) levels were measured by blood cell counter (Sysmex KX-21N, Japane). Separated serum and whole blood were kept frozen at -80°C until analysis. Sera samples were analysed using an auto-analyser (BT3000, Italy) and manufacturer's reagent kits for serum biomarkers such as high sensitivity C-reactive protein (hs-CRP), total iron binding capacity (TIBC), iron, calcium, phosphorus, magnesium, urea, creatinine, albumin and total protein. Serum hepcidin and ferritin levels were assayed by standard enzyme linked immunosorbent assay (ELISA) kits (Cusabio and DiaPlus, China, USA, respectively). Serum concentrations of interleukin 6 (IL-6) were measured using human ELISA kits according to the manufacturer's instructions (Bender Med Systems, Vienna, Austria). These biochemical factors were assayed using fully automated ELISA analyser (TKA Teknolabo Bizet, Italy). Serum PTH levels were measured through a chemiluminescent method (Liaison, USA).

The collected data was analysed using statistical methods such as Pearson's Correlation Coefficient and Scatter plot. P value less than 0.05 was considered statistically significant.

Results

There were 40 patients among whom 15(37.5%) were males and 25(62.5%) were females with an overall mean age of 55.68±14.4 years. There was a significant inverse relationship between serum hepcidin concentrations and Hb levels ($p<0.05$). No significant relationship between serum iron and serum hepcidin was found ($p>0.05$). An inverse significant correlation between serum levels of hepcidin and TIBC was observed ($p<0.001$). Hepcidin had an inverse significant relationship with albumin and PTH levels ($p<0.05$). There was a positive significant relationship of hepcidin serum levels with IL-6 and ferritin serum levels ($p<0.05$ each). There was a significant positive correlation between Hb and ferritin levels ($p<0.05$ each). There was no significant relationship of hepcidin with serum hs-CRP and GFR.

Discussion

Anaemia is one of the major complications of CKD and its pathogenesis has not been well understood.[13] Inflammation is an important factor associated with erythropoietin resistance and the occurrence of anaemia in patients with CKD.[14] It has been shown that hepcidin expression is part of an inflammatory reaction and it may be involved in the pathogenesis of inflammation-associated anaemia.[15] In our study, serum hepcidin concentrations were negatively associated with Hb levels and it had strong positive correlation with IL-6 levels. Our results are inconsistent with the results of some studies in CKD patients. Studies in non-dialysis CKD patients could not find association between serum hepcidin concentration and Hb level.[5,16] A study comprising renal insufficiency patients also could not find any relation between Hb levels and serum prohepcidin concentrations.[17] Our results are consistent with the results of other studies in dialysis patients and in

Table-1: Clinical characteristics.

Parameter	Mean ± SE (Mean ± SD)	Parameter	Mean ± SE (Mean ± SD)	Parameter	Mean ± SE (Mean ± SD)
Hepcidin (ng/ml)	258.86 ± 21.94 (258.86 ± 138.76)	hs-CRP (mg/l)	10.54 ± 1.89 (12.82 ± 13.74)	Creatinin (mg/dl)	3.66 ± 0.15 (3.66 ± 0.96)
IL-6 (pg/ml)**	0.59 ±3.39 (5.57 ± 9.25)	TIBC (µg/dl)*	295.05 ± 8.18 (295.05 ± 51.79)	Phosphorus (mg/dl)	4.36 ± 0.12 (4.36 ± 0.77)
Haemoglobin (g/dl)*	10.01 ± 0.24 (10.01 ± 1.56)	Hematocrit (%)	31.15 ± 0.69 (31.15 ± 4.39)	Weight (kg)	72.4 ± 1.73 (72.40 ± 10.95)
GFR (ml/min/1.73 m2)	21.51 ± 0.74 (21.51 ± 4.71)	Urea (mg/dl)	126.38 ± 7.12 (126.37 ± 45.02)	PTH (pg/ml)*	111.93 ± 13.35 (152.11 ± 146.10)
Ferritin (µg/l)**	163.83 ± 24.69 (241.06 ± 276.36)	Albumin (g/dl)*	4.21 ± 0.11 (4.21 ± 0.74)	Magnesium (mg/dl)	2.01 ± 0.04 (2.01 ± 0.29)
Iron (µg/dl)**	63.5 ± 4.64 (63.50 ± 29.39)	Total protein(g/dl)	8.11 ± 0.17 (8.11 ± 1.07)	Calcium (mg/dl)	9.03 ± 0.11 (9.03 ± 0.75)

TIBC= Total Iron Binding Capacity, IL-6= interleukin-6, PTH= parathyroid hormone, GFR= Glomerular filtration rate.

*An inverse significant relationship with hepcidin in Pearson Correlation ($p<0.05$).

**A positive significant relationship with hepcidin in Pearson Correlation ($p<0.05$).

patients with both heart and kidney failure.[15,18] These conflicting results may be attributable to differences in the iron status of the populations studied, differences in inflammatory state or sample size. There are several reasons why hepcidin may be negatively associated with Hb in stage 4 CKD patients with sufficient iron stores.[19] For one, hepcidin as central modulator of iron controls the expression of ferroportin (the major transporter of iron) on intestinal cells and macrophages and limits iron availability for erythropoiesis.[20] High-ferritin levels in serum normally indicate iron overload, but this does not necessarily mean sufficient bone marrow iron stores.[21] Another reason is that hepcidin is induced by inflammation,[2] and patients with CKD have a chronic inflammatory state.[3] The effects of inflammation on the synthesis of hepcidin are well understood and are mediated at least in part by IL-6. The third reason is that besides inducing iron-restricted erythropoiesis, hepcidin directs inhibitory effects on erythropoiesis and affects erythroid precursor proliferation and survival.[22] Lastly, diminished renal clearance in CKD patients also elevates hepcidin serum concentration and exacerbates its effects.[4] Thus, hepcidin excess has emerged as one of the key pathogenic features of inflammation-associated anaemia and reduction of Hb levels in stage 4 CKD patients.

We observed a significant positive correlation between hepcidin and ferritin as widely used marker of iron status.[23] In agreement with our study, it has been shown that IL-6 and other inflammatory cytokines may change in concentrations of not only serum hepcidin, but also iron status markers.[24] A study in patients with inflammation-associated anaemia reported that hepcidin level correlates well with the serum ferritin concentration.[25] A study in haemodialysis patients described that hepcidin level correlates with TIBC and ferritin concentrations.[26] One study in patients with chronic liver disease showed that hepcidin level correlated with ferritin level.[27] In line with these and other findings,[26,28] our results suggest that synthesis of hepcidin is also enhanced by an increased body iron store. A significant positive correlation between hepcidin and ferritin indicates the association between hepcidin and iron stores in stage 4 CKD patients. Our study showed that the raised hepcidin in stage 4 CKD patients may be due to elevated ferritin levels and elevated IL-6 due to inflammation status.

The low number of patients with stage 4 CKD and removal of subjects based on the inclusion/exclusion criteria were limitations of our study.

Conclusions

Hepcidin had negative correlation with Hb level in stage 4 CKD patients with adequate iron stores, which could be effective in the development of anaemia in these patients. Further studies are needed to examine longitudinal changes in hepcidin, Hb, iron status indicators and inflammatory cytokines such as IL-6 in stage 4 CKD patients.

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